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Caves as islands: mitochondrial phylogeography of the cave-obligate spider species *Nesticus barri* (Araneae: Nesticidae)

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Abstract. Around 1000 obligate eave species have been described from the continental United States. This taxonomically diverse group of species eontains both terrestrial obligate cave species (troglobites) and aquatic obligate cave species (stygobites). The greatest diversity of troglobites in the United States occurs on the southern Cumberland Plateau in south-central Tennessee and northeastern Alabama. The troglobitic spider *Nesticus barri* Gertsch 1984 is known from nearly 60 caves in this area. We studied the mitochondrial phylogeographic structuring of this species, sampling individuals from twelve caves across the species' range. We found that *N. barri* populations within individual caves are generally not genetically diverse; that *N. barri* is divided into genetically distinct subpopulations, with mitochondrial cytochrome oxidase I genetic distances between subpopulations ranging from 0.021 to 0.045; and that female-based migration between caves is minimal or nonexistent, even over small geographic scales (< 15 km). This is the first genetic study of a troglobitic taxon from this biodiverse region. Our results contrast with those from previous studies on stygobitic crayfish from this area, which showed high levels of gene flow between caves.

Keywords: Cumberland Plateau, troglobite, gene flow, mitochondria, cytochrome oxidase I

Caves are home to a unique and diverse community of species. Species that complete their life cycles within caves and are never found outside of caves are known as 'obligate cave species', and around 1000 sueh species have been described from the continental United States (Culver et al. 2000). The dominant taxonomic groups are arachnids, crustaceans, and hexapods, but also known are mollusks, diplopods, fish, and salamanders (Culver et al. 2000). Terrestrial obligate cave species are known as troglobites and aquatic obligate cave species are referred to as stygobites. Cave obligate species have often evolved in a convergent manner such that most have small to absent eyes, are light colored, and have long appendages, a condition referred to as troglomorphy (reviewed in Porter 2007).

Obligate cave species are not distributed evenly across the continental United States. Fewer than one fifth of all counties have even one troglobite or stygobite (Culver et al. 2000), whereas a few areas have an exceptionally diverse cave fauna. For troglobites, the highest diversity is found on the southern Cumberland Plateau in northeastern Alabama and southern Tennessee. The Cumberland Plateau is one of the largest karst regions in the eastern United States, and is exceptionally cave-rich (Christman and Culver 2001). Culver et al. (2000) found that Jackson County in northeastern Alabama has more troglobitic species (52) than any other county in the United States, and that Marshall County, to the south of Jackson County, had the fourth most species of troglobites (32). Recent surveys in southern Tennessee found similarly high levels of troglobitic diversity, with Franklin County having 34 species of troglobites, and Marion County 24 species (Culver et al. 2000; Lewis 2005).

Cave habitats, as compared to surface habitats, are limiting in that cave species are often restricted in where they can live and their ability to move between habitats (caves). Accordingly, many troglobites have extremely small ranges, with nearly 70% of troglobitic species and subspecies limited to a single county, and many species known from a single cave (Culver et al. 2000). Troglophiles, which are able to survive outside caves, though they tend to complete their life cycle within caves, and trogloxenes (such as bats), which do not complete their life cycle in caves but often use them for shelter, usually have larger ranges and higher levels of gene flow because of greater continuity between habitats (Caccone 1985).

The only genetic studies on the troglobitic or stygobitic fauna of the southern Cumberland Plateau were conducted by Buhay and Crandall (2005) and Buhay et al. (2007) on two genera of stygobitic crayfish (*Orconectes* and *Cambarus*). They found that these crayfish have large population sizes, high genetic diversity, and extensive gene flow between caves as evidenced by haplotypes shared among multiple caves across several counties (Buhay and Crandall 2005; Buhay et al. 2007). Contrasting results have been found in studies of troglobites from other areas, including several species of troglophilic and troglobitic spiders of the Appalachians (Hedin 1997a), which showed significant population structure and little evidence for migration between caves. This difference may be due to the generally broader connections present between subterranean aquatic habitats than between subterranean terrestrial habitats (Porter 2007).

Spiders of the genus *Nesticus* are diverse in the southeastern United States, where at least 30 different species occur in the southern Appalachian Mountains and Cumberland Plateau (Gertsch 1984; Hedin 1997b; Hedin and Dellinger 2005). These medium-sized (2 to 7 mm) spiders are limited to cool, moist microhabitats in the southeastern United States. About one-third of this regional fauna includes troglophilic or troglobitic species (Hedin and Dellinger 2005).

In addition to taxonomic studies, several studies have been condueted on *Nesticus* spiders. Hedin (1997b) studied the phylogenetic history of the *Nesticus* species of the southern Appalachian Mountains and population genetics of the

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Table 1.—Summary of genetic samples – including cave sites, sample sizes, haplotype identities, and network inclusion information.

Network-cave	County	State	Number of individuals	Haplotype
A- Sewanee Blowhole	Franklin	Tennessee	3	A
B- Salt River Cave	Jackson	Alabama	2	B
C- White Cricket Cave	Marion	Tennessee	5	C1 (×4), C2 (×1)
C- Tate Spring	Marion	Tennessee	2	C3
D- Lost Cove Cave	Franklin	Tennessee	3	D
E- Lost Cove Cave	Franklin	Tennessee	5	E1
E- Keith Cove Cave	Franklin	Tennessee	5	E2
E- Grapeville Cave	Franklin	Tennessee	3	E2 (×2), E3 (×1)
F- Guess Creek Cave	Jackson	Alabama	4	F1
F- Gross Skeleton Cave	Jackson	Alabama	1	F2
F- Bishop Cave	Marshall	Alabama	3	F3
G- Tate Cave	Jackson	Alabama	6	G1 (×3), G2 (×2), G3 (×1)
G- Jess Elliot Cave	Jackson	Alabama	2	G2

Nesticus tennesseensis complex (1997a). This species complex is found in eastern Tennessee, western North Carolina, western Virginia, and southern West Virginia. He found a small number of closely-related haplotypes in each individual cave population, whereas haplotypes between populations were divergent, indicating that these populations last shared a common ancestor a relatively long time ago. As no haplotypes were shared between populations, there is evidently little to no gene flow between populations of these spiders (Hedin 1997a).

Nesticus barri Gertsch 1984 is a troglomorphic (pale, eyeless, and long-limbed) species known from around 60 caves across the hotspot of troglobite diversity in Jackson and Marshall Counties, Alabama, and Franklin and Marion Counties, Tennessee (Gertsch 1984; Hedin and Dellinger 2005; Lewis 2005). They spin webs that act as both a home and a means to catch prey. They hang upside down from their webs and do not stray far from them throughout their lives (Gertsch 1984; Hedin 1997b). Female spiders carry their egg sacs on their spinnerets until the offspring hatch (Reeves 1999). On the basis of morphology, Hedin and Dellinger (2005) synonymized *N. valentinei* Gertsch 1984, a species known from only one cave on the edge of *N. barri's* range, with *N. barri* (Gertsch 1984). Previous molecular work with *N. barri* was limited to four individuals that were sequenced for phylogenetic analysis by Hedin (1997b).

The objective of this study was to examine the mitochondrial phylogeographic structuring of *N. barri*. We gathered specimens from caves across the range of the species to determine levels of genetic diversity within individual caves and across the range of the species, and to determine how much gene flow occurs between caves. We hypothesized that there would be little to no gene flow between caves. We also gathered genetic evidence to support or reject the synonymization of *N. valentinei* with *N. barri*. Our study constitutes the first to examine genetic structuring of a troglobite from the southern Cumberland Plateau.

METHODS

Samples.—Forty-five specimens were obtained from twelve different caves (Table 1) that spanned the range of *Nesticus barri* (Figs. 1, 2). Individuals were preserved in the field in 95% ethanol and taken back to the laboratory where they were stored at −80° C. We report, for the first time, the presence of *N. barri* in Grapeville Cave and Sewanee Blowhole in Franklin County, Tennessee, USA; these are the northernmost records for this species. To protect sensitive cave habitats, cave locations are referred to only by Tennessee and Alabama Cave Survey names and by approximate locations on maps; detailed collection information can be obtained from the authors.

DNA extraction, amplification, and sequencing.—DNA was extracted using the tissue from one leg of small individuals or the femur of large individuals aceording the manufacturer's instructions for the DNeasy Blood and Tissue Kit (Qiagen; P/N: 69506). Initial polymerase chain reaction (PCR) amplifications for part of the mtDNA cytochrome oxidase I (COI) gene were done using the primers LCOI (5′-GGTCAACAAATCATAA-AGATATTG-3′) and HCOI (5′-TAAACTTCAGGGTGAC-CAAAAAATCA-3′) from Folmer et al. (1994). We later developed a species-specific replacement for the LCOI primer that was more effective in *N. barri* (LCOI-barri; 5′-GGACTT-TGTATTTTATTCTTGGGTC-3′). Two different polymerase enzymes were used: Amplitaq Gold PCR Master Mix (Applied Biosystems; P/N: 4318739) or Taq DNA Polymerase (Sigma; P/N: D5938). When Taq DNA Polymerase was used, PCR conditions were 1 min at 94° C, 2 min at 50° C, and 90 s at 72° C (× 35 times). When Amplitaq Gold PCR Master Mix was used, the conditions were 5 min at 95° C, followed by 35 cycles of 15 s at 95° C, 15 s at 50° C, and 1 min at 72° C. Successful PCR reactions were purified according to the manufacturer's instructions for the QIAquick PCR Purification Kit (Qiagen; P/N: 28106). Sequencing reactions on both strands were performed by the DNA Analysis Facility at Yale University and were analyzed on an Applied Biosystems 3730 sequencer. The resulting sequences were edited using Sequencher (v. 4.9; Gene Codes Corp., Ann Arbor, MI). Sequences have been submitted to GenBank (Accession #GQ421645-GQ421688).

Intraspecific analyses.—No indels were present, and sequences were aligned by eye. Numbers of variable sites, transitions, transversions, and predicted amino acid changes were determined in Mesquite (v. 2.6; http://mesquiteproject.org). The

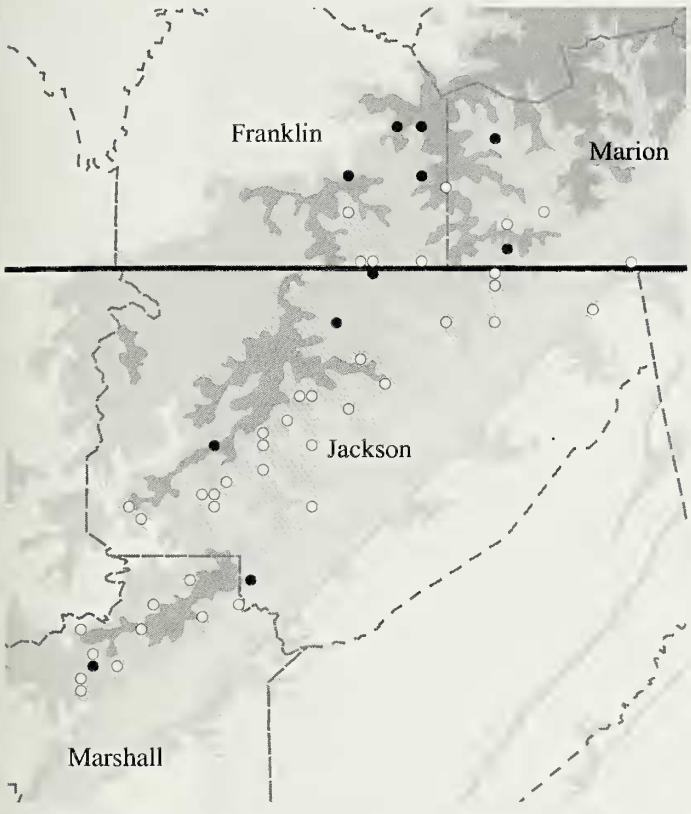

Figure 1.—Extent of the Cumberland Plateau in southern Tennessee and northeastern Alabama and distribution of *Nesticus barri*. The top of the Cumberland Plateau is indicated in dark grey, and lower elevations are indicated by lighter shades. Approximate location of caves in which *Nesticus barri* has been found (all circles) and caves which we sampled (filled circles). Tate Cave and Jess Elliot Cave, whose entrances are close together, are indicated by a single circle.

number of haplotypes present was determined by combining identical sequences, including those that differed only in length at one end of the sequence or by one or more ambiguous bases. We used TCS (v. 1.21; Clement et al. 2000) to group these haplotypes into networks. We used PAUP* (v. 4.0b10; Swofford 2001) to calculate mean uncorrected 'p' distances between haplotypes both within and between TCS networks. We also examined population structure by calculating *F*-statistics in Arlequin (v. 2.0; Schneider et al. 2000) among the caves where we sampled four or more individuals (Table 1).

Phylogenetic analyses.—To test the monophyly of *N. barri*, and to allow us to compare intraspecific diversity within *N. barri* with interspecific diversity between *N. barri* and other *Nesticus* species, we used partially overlapping COI sequences from nine other nesticids. These included sequences from seven other *Nesticus*, and two sequences from the more distantly-related *Eidmanella pallida* (Emerton 1875) (GenBank Accession #GQ421636-GQ421644). Six of the seven *Nesticus* species included here are found in the same geographic area as *N. barri* (Gertsch 1984; Hedin and Dellinger 2005); *N. silvestrii* Fage 1929 is found in the Pacific Northwest, and is an outgroup to the *Nesticus* species of the Appalachians (Hedin 1997b).

We used MrBayes (v. 3.1.2, Ronquist and Huelsenbeck 2003) to conduct Bayesian phylogenetic analyses on a matrix of all sequences (both ingroup and outgroup). We partitioned the data by codon position, and for each partition we used a General Time Reversible (GTR) model with six substitution rates, estimated nucleotide frequencies, and invariable sites. These model parameters were unlinked between partitions with the exception of substitution rates, which were linked for the 1st and 2nd codon position partitions due to the small number of changes in the 2nd codon position. We calculated clade credibility values from 4000 trees by sampling every 1000th tree from two runs of 5,000,000 trees after discarding the first 3001 sampled trees of each run. We used AWTY (Wilgenbusch et al. 2004) to confirm stationarity and convergence of the Bayesian analyses.

We also conducted a distance-based neighbor-joining bootstrap analysis (1000 replicates) in PAUP*. We used Modeltest (v. 3.7; Posada and Crandall 1998) to identify the model that best described the evolution of the sequences (as selected by the Akaike Information Criterion; Posada and Buckley 2004) and used the parameters identified in Modeltest (GTR + Γ + invariable sites) in distance analyses.

RESULTS

Molecular evolution within *N. barri*.—Amplification and sequencing were successful for 44 of 45 *N. barri* specimens. Sequences ranged in length from 598 to 633 bp, with a mean length of 628 bp. No indels were observed. Seven ambiguous nucleotides were present in 27,632 bp of sequence gathered from *N. barri*. Disregarding ambiguous nucleotides, there were 53 variable sites in the *N. barri* dataset. Forty-six of these sites varied by a transition substitution, five by a transversion substitution, and two sites exhibited both transition and transversion substitutions. Based on translations of the nucleotide sequences, nine of 211 amino acids were predicted to be variable. No stop codons were observed within any translated amino acid sequence.

Population structure.—Among the 44 sampled individuals we identified fifteen haplotypes (Table 1). Thirteen of these haplotypes were found in a single cave, and two haplotypes were shared between geographically-adjacent caves. Individual cave samples were generally not genetically diverse; eight caves were fixed for a single haplotype, three caves had two haplotypes, and one cave had three haplotypes. In three of the cases where a single cave had multiple haplotypes, those haplotypes differed by one or two nucleotides. In one exceptional case (Lost Cove Cave) two haplotypes were present and these haplotypes differed by 14 nucleotides.

The fifteen *N. barri* haplotypes fell into seven unconnected haplotype networks based on the 95% parsimony probability (Templeton et al. 1992), which separated networks that differed by more than ten nucleotides (Fig. 3). Several 'networks' contained only a single haplotype (A, B, D), and no network contained more than 3 haplotypes (Fig. 3). Most haplotypes within a network differed by a single nucleotide, with one network (F) containing three haplotypes that differed by as many as three nucleotides, and another (C) containing three haplotypes that differed by as many as seven nucleotides (Figure 3). No cave contained haplotypes from more than one network with the exception of Lost Cove Cave, with one haplotype from each of network D and E (Table 1). Mean pairwise genetic distances between haplotypes from unconnected networks ranged from 0.021 to 0.045 (uncorrected 'p' distance; Table 2). Mean pairwise distances between haplotypes

Figure 2.—*Nesticus barri* female in web, Marlow Holes, Franklin County, Tennessee. Photo by Alan Cressler.

within a network ranged from zero (for those networks containing a single haplotype) to 0.007 (Table 2).

The seven genetic networks are also largely geographically continuous. Caves with spiders from a single network are in the same area (Fig. 4). The greatest geographic distance between caves containing spiders with haplotypes from the same network was 37 km in network F (Fig. 4). The closest that we found spiders from two different networks were the two found in Lost Cove Cave (Fig. 4, Table 1). This is exceptional, as all other caves had haplotypes from a single network. The two cases where haplotypes were shared between caves involve caves that are geographically proximate: haplotype G2 in Tate Cave and Jess Elliot Cave (entrances less than 0.5 km apart), and haplotype E2 in Keith Cove Cave and Grapeville Caves (entrances ~11 km apart).

We tested for population structure among all caves where we sampled four or more individuals (Table 1), using F statistics. For the nine comparisons involving caves whose spiders were from different haplotype networks, F_{ST} values ranged from 0.72 to 1.00 and were significant ($P < 0.01$). For the comparison between Keith Cove Cave (with spiders with haplotype E2, Table 1) and Lost Cove Cave (haplotypes D and E1, Table 1), the F_{ST} value was 0.24 ($P = 0.037$).

Phylogeny.—Despite the relatively short length of the sequences we gathered, the phylogenetic tree constructed from the *N. barri* haplotypes, representative sequences from seven *Nesticus* species, and two *Eidmanella* individuals, was largely congruent with the phylogeny for the Appalachian *Nesticus* species previously reported by Hedin (1997b) (Fig. 5). We identified *N. silvestrii* as the sister group to the Appalachian *Nesticus* species, *N. archeri* Gertsch 1984, *N. pecki* Hedin & Dellinger 2005, and *N. stygius* Gertsch 1984 as early-diverging, and *N. barri* most-closely related to *N. furtivus* Gertsch 1984, *N. georgia* Gertsch 1984, and *N. jonesi* Gertsch 1984. We found support (Bayesian clade credibility value of 91%; Fig. 5) for the monophyly of *N. barri*. Within *N. barri*, we identified seven primary lineages (A–G) that correspond directly to the haplotype networks (Fig. 3). When these lineages contained more than one haplotype (lineages C, E, F, and G; Fig. 5) we found strong support for their monophyly. It is notable that relationships among the seven primary lineages of *N. barri* are poorly resolved (Fig. 5), with no sister-lineage relationships being strongly supported in either Bayesian or neighbor-joining analyses.

DISCUSSION

The caves of a four-county area spanning the Tennessee/Alabama state line are inhabited by more species of troglobites than any other known comparable area in the United States. Despite this great species diversity, no detailed genetic studies have been conducted on any troglobite from this area. *Nesticus barri* is a troglobitic spider that is known from caves across this area. The objectives of this study were to examine mitochondrial genetic diversity and population structure in *N. barri*, to support or reject the recent synonymization of *N. valentinei* with *N. barri*, and to compare the results from *N. barri* to previous studies on stygobites from this area. This study also represents an additional step towards building a comparative molecular phylogeographic perspective for the diverse cave-obligate fauna of the region.

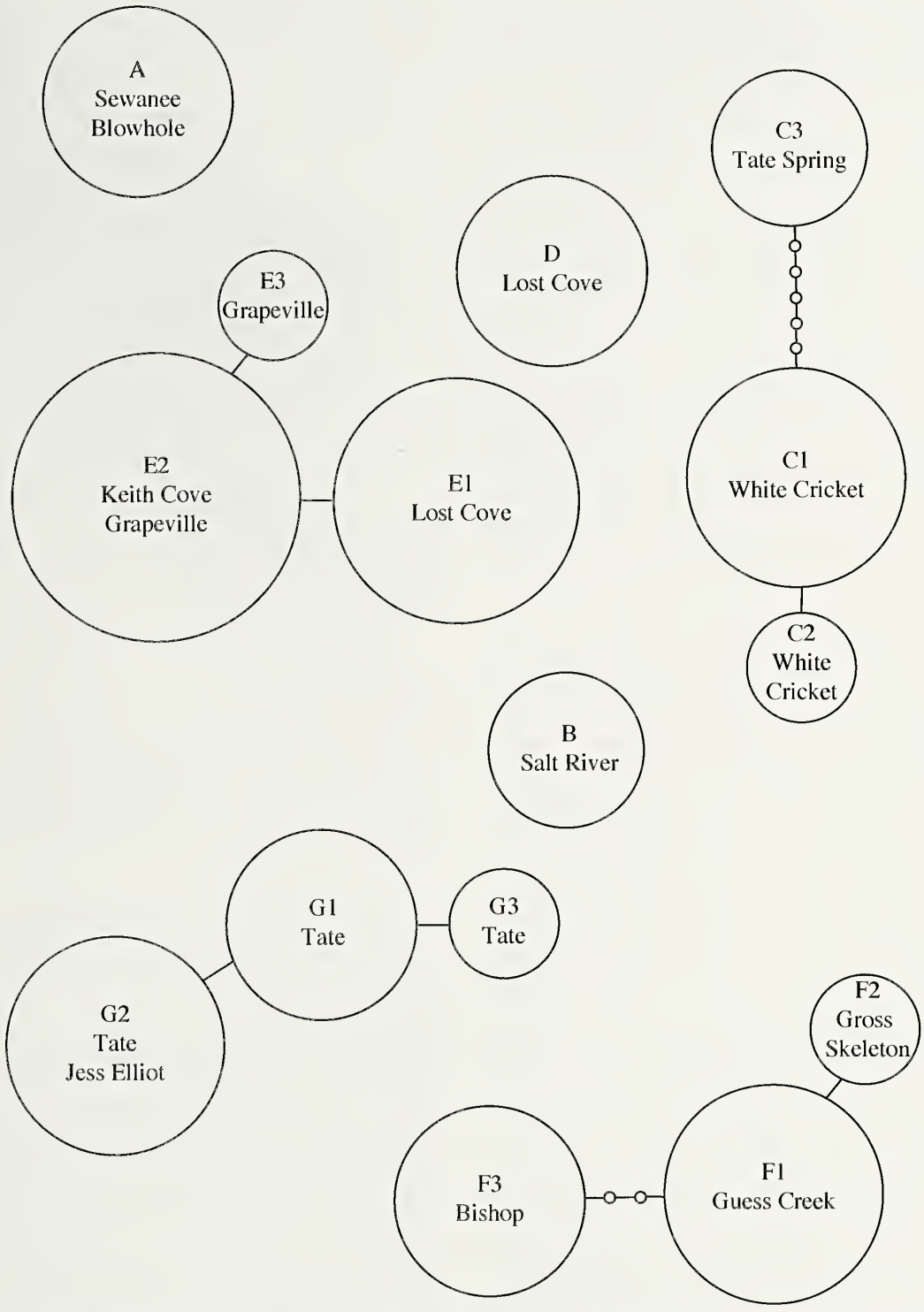

Figure 3.—Haplotype networks for *N. barri* COI, with locality information for each haplotype. The distribution of networks roughly corresponds to their geographic location (see Figure 4 for more details). The area of each circle is proportional to the number of sampled individuals with each haplotype. Unsampled and/or extinct haplotypes are indicated by small open circles. Unconnected networks differ by more than ten nucleotide substitutions. Lost Cove Cave is the only cave with individuals present in two different networks.

Genetic diversity and population structure in *N. barri*.—The significant genetic diversity found within *Nesticus barri* is partitioned into a number of geographically distinct subpopulations. We found no examples of shared haplotypes between caves that were more than 12 km apart and no examples of shared mitochondrial lineages at distances over 40 km (Figs. 4, 5). Given the general lack of shared haplotypes between caves it is not surprising that all F_{ST} comparisons showed significant population structure. We found complete congruence between the haplotype networks and primary Bayesian mitochondrial lineages (Figs. 3, 5). The mitochondrial lineages found in *N. barri* differ from one another by 2.1–4.5%,

Table 2.—Mean pairwise uncorrected 'p' distances between haplotypes within networks (on diagonal, in italics) and haplotypes from different networks (below diagonal).

	A	B	C	D	E	F	G
A	*0.000*						
B	0.027	*0.000*					
C	0.024	0.031	*0.007*				
D	0.021	0.025	0.023	*0.000*			
E	0.032	0.028	0.029	0.022	*0.002*		
F	0.042	0.032	0.041	0.034	0.033	*0.004*	
G	0.036	0.036	0.045	0.028	0.027	0.037	*0.002*

indicating isolation for significant periods of time (Table 2). Remarkably, caves less than 10 km apart may have spider populations with mitochondrial haplotypes that were placed into different haplotype networks. The observation that haplotypes were typically unique to a single cave (Table 1) indicates that there is currently little to no migration of spiders between caves. The observation that highly distinct mitochondrial lineages occupy geographically adjacent areas yet have not mixed suggests that migration has been limited for a very long time.

One notable result was the presence of spiders with haplotypes from two different networks (E and D; Fig. 3) in Lost Cove Cave. All of these spiders were collected on the same date, and within 100 m of one another. Spiders from network E (though with different haplotypes) were found in two other caves near Lost Cove Cave, whereas we did not collect spiders from network D from any other cave (Fig. 3). It is unclear whether the presence of spiders with significantly different haplotypes in this cave is a result of mixing due to migration, or to long-term coexistence and divergence.

Our results are consistent with the preliminary studies of *N. barri* by Hedin (1997b). Hedin (1997b) performed a TCS analysis on haplotypes from a mitochondrial region spanning partial 16S, complete tRNA-leucine, and partial NADH dehydrogenase subunit I genes from four *N. barri* individuals (one each from Salt River Cave, Lost Cove Cave, Bishop Cave, and Guess Creek Cave). Hedin (1997b) found that only the individuals from Bishop Cave and Guess Creek Cave were joined in a network. We found, similarly, that individuals from those two caves have CO1 haplotypes belonging to network F (Table 1), whereas spiders from the other two caves (Salt River and Lost Cove) belonged to distinct networks (B, and either D or E, respectively; Table 1).

Status of *N. valentinei*.—Gertsch (1984) described *Nesticus valentinei* as a new species from Heating Stove Cave in Marion County, Tennessee, on the northeastern edge of *N. barri*'s range. The entrance to Heating Stove Cave was evidently destroyed during the construction of an interstate highway (Hedin and Dellinger 2005). Hedin and Dellinger (2005) collected specimens from Tate Spring, which is presumably connected to Heating Stove Cave. These individuals were morphologically compared to other *N. barri* individuals and to the *N. valentinei* holotype; based on this comparison, these authors concluded that *N. valentinei* was not distinct from *N. barri*, and accordingly synonymized *N. valentinei* with *N. barri*.

We found that individuals from Tate Spring, though they exhibited a unique haplotype ('C3' in Table 1 and Fig. 5), are nested within *N. barri*, most closely related to specimens from nearby White Cricket Cave (Table 1, Figs. 3, 4). As such, our results provide independent corroboration of the synonymization of *N. valentinei* with *N. barri* as proposed by Hedin and Dellinger (2005).

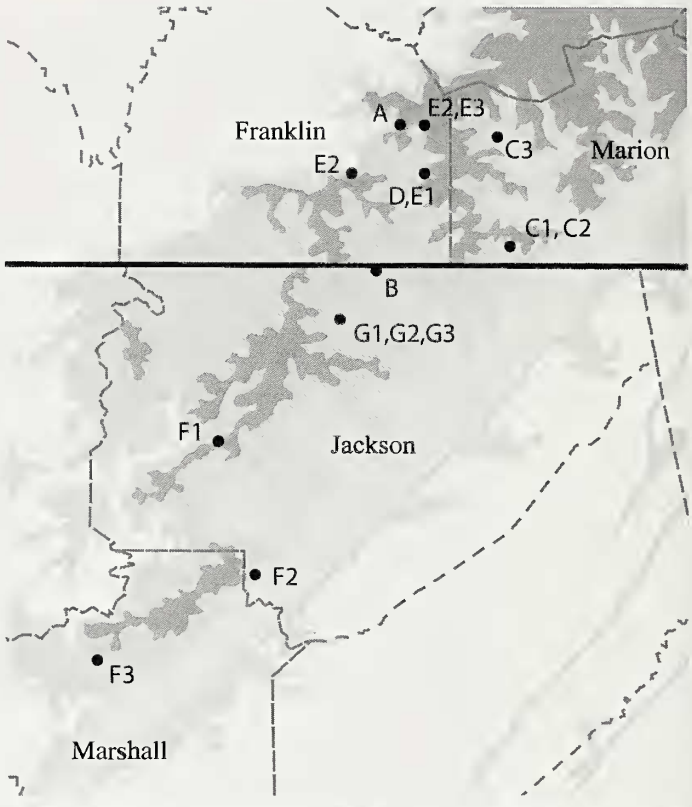

Figure 4.—Distribution of COI haplotypes and networks in *N. barri*. Haplotype labels correspond to those provided in Table 1. Approximate locations of sampled caves are indicated by black filled circles. Tate Cave and Jess Elliot Cave, whose entrances are close together, are indicated by a single filled circle (with haplotypes G1, G2, and G3).

Population structure of *Nesticus* from other areas and of stygobites of the Cumberland Plateau.—Because there have been no genetic studies on troglobites from the Cumberland Plateau, we are limited to comparing our results to studies on *Nesticus* species from other regions, and to stygobites from the Cumberland Plateau. Our findings in *N. barri* are consistent with those of Hedin (1997a) for the *Nesticus tennesseensis* complex. This complex contains seven species, some of which are surface species, some troglophilic, and some troglobitic (Hedin 1997a). Hedin (1997a) found that there was little to no mitochondrial gene flow between populations of these spiders, regardless of their habitat requirements. He also found significant genetic divergence on a small geographic scale. Cesaroni et al. (1981) examined three species of *Nesticus* in Italy using isozymes and found that genetic diversity was slightly less in the two cave dwelling species than in the surface dwelling species that they studied.

As *N. barri* is restricted to cave environments, these caves are effectively 'islands' of habitat. Though the southern Cumberland Plateau has one of the highest cave densities in the eastern United States (Christman and Culver 2001), significant genetic diversity is present across the range of this

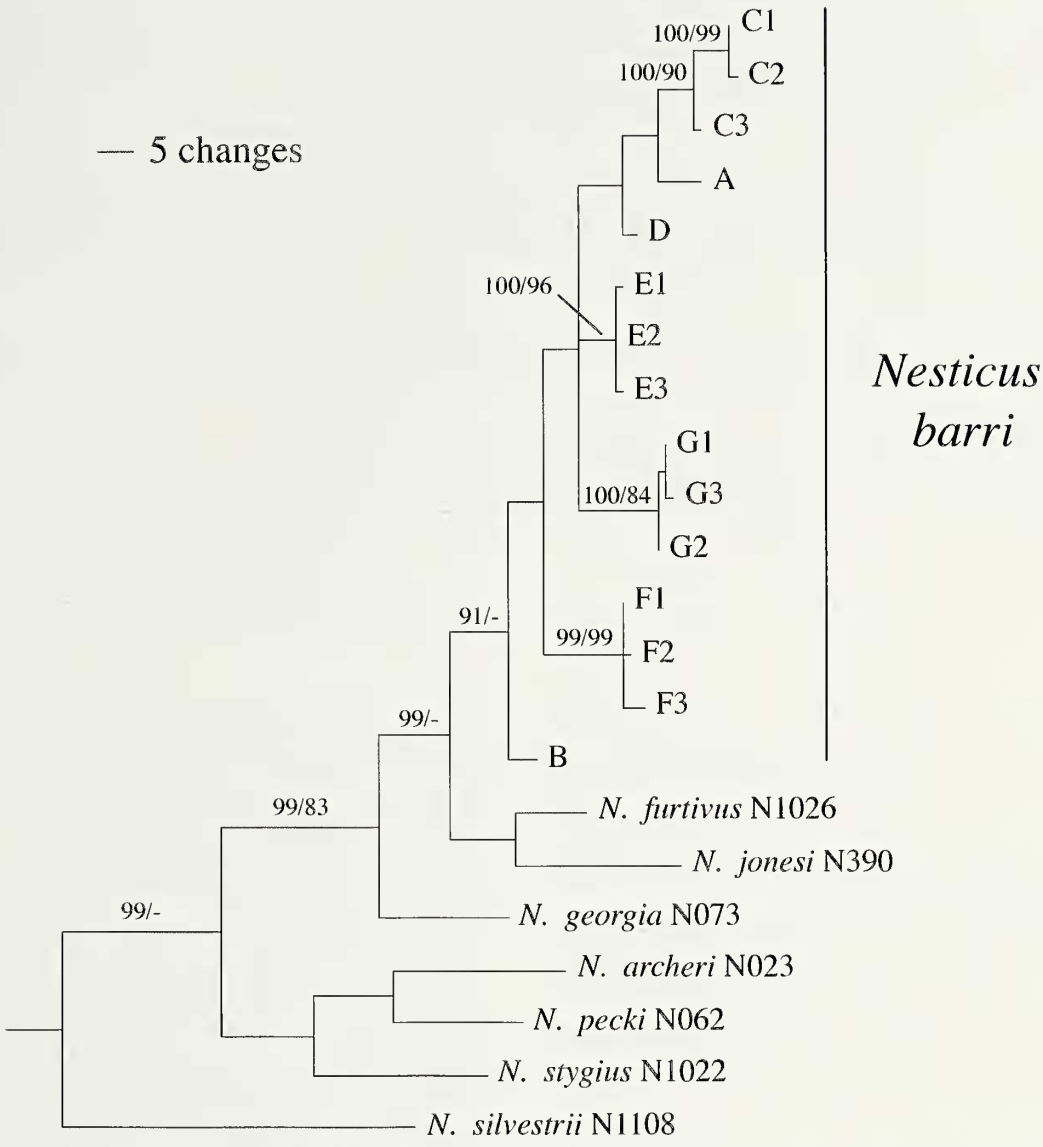

Figure 5.—Bayesian majority rule consensus tree based on cytochrome oxidase I sequences. *Nesticus barri* haplotypes correspond to those in Table 1. The tree is rooted with sequences from *Eidmanella* (not shown). Bayesian clade credibility values (from 4000 trees) greater than 90%, and neighbor-joining bootstrap values (from 1000 replicates) greater than 80%, are indicated above branches.

species, indicating that even with other 'islands' nearby, these spiders rarely migrate from one to another. Further studies are necessary to determine whether other troglobitic taxa (e.g. beetles, millipedes, flatworms) maintain population connectivity across the habitat 'islands' of the southern Cumberland Plateau.

The results in *N. barri* contrast with the two population genetic studies on cave crayfish whose ranges extend into the southern Cumberland Plateau. For both *Orconectes australis* and *Cambarus hamulatus* there was genetic evidence for large population sizes and extensive gene flow among caves (Buhay and Crandall 2005; Buhay et al. 2007). Greater population connectivity in both of these species was also evident in their haplotype networks, where a single network included all members of the species (Buhay and Crandall 2005; Buhay et al. 2007). Stygobites, such as these crayfish, may have a higher rate of gene flow between populations because they can migrate through underground aquifers that are inaccessible to troglobites (Porter 2007).

CONCLUSION

Nesticus barri shows significant genetic diversity on a small geographic scale. Female-based migration between caves appears to be extremely limited. The isolation and diversity of populations of *N. barri* has conservation implications because the loss of a single population or of several nearby populations could mean the loss of a distinct genetic lineage. This pattern contrasts with that found for stygobites from the same area, which show evidence for gene flow over significant distances. Further studies on other troglobitic taxa in this biodiverse region will clarify whether the pattern of population structure observed in *N. barri* is common, or whether it is unique to this cave spider.

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